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Antioxidant and Antimicrobial Effects of *Trametes versicolor* (L.) Lloyd Extracts in Different Solvents

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Abstract: *Trametes versicolor* (L.) Lloyd known as turkey tail, is a medicinal mushroom belonging to the Polyporaceae. Although the consumption and commercial sale of this mushroom in our country is new, it has been used for centuries as a medicine in some countries, especially in China. In this study, it was aimed to determine the antimicrobial and antioxidant effects of ethanol and methanol extracts of *T. versicolor*. Its antimicrobial effects were determined by disk diffusion and microdilution method using pathogenic microorganisms such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus megaterium*, *Staphylococcus aureus*, *Candida albicans* and *Trichophyton* sp. Total antioxidant level, total oxidant level and DPPH radical scavenging capacity were detected for the antioxidant activity of the mushroom. According to the results obtained, it was seen that these extracts inhibit the growth of microorganisms at different rates (10-21 mm) according to the disk diffusion method. The minimal inhibitory concentrations of *T. versicolor* against microorganisms used were determined to be between 62.5-250 µg/mL. The TAS and TOS values of the methanol extract were 0.72 mmol Trolox Equiv./L and 18.39, respectively, the TAS and TOS values of the ethanol extract were detected 0.88 mmol Trolox Equiv./L and 16.71 µmol H_2O_2 Equiv./L, respectively.

Key words: Medicinal mushrooms, *Trametes versicolor*, antimicrobial, antioxidant.

Trametes versicolor (L.) Lloyd'un Farklı Çözücülerdeki Ekstraktlarının Antioksidan ve Antimikrobiyal Etkileri

Öz: Hindi kuyruğu olarak bilinen *Trametes versicolor* (L.) Lloyd, Polyporaceae familyasına ait tıbbi bir mantardır. Bu mantarın ülkemizde tüketimi ve ticari satışı yeni olmasına rağmen Çin başta olmak üzere bazı ülkelerde yüzyıllardır ilaç olarak kullanılmaktadır. Bu çalışmada, T. versicolor'un etanol ve metanol ekstraktlarının antimikrobiyal ve antioksidan etkilerinin belirlenmesi amaçlanmıştır. Antimikrobiyal etkileri, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus megaterium*, *Staphylococcus aureus*, *Candida albicans*, *Trichophyton* sp. gibi patojenik mikroorganizmalar kullanılarak disk difüzyon ve mikrodilüsyon yöntemi ile belirlendi. Mantarın antioksidan aktivitesi için toplam antioksidan seviyesi, toplam oksidan seviyesi ve DPPH radikal süpürme kapasitesi tespit edildi. Elde edilen sonuçlara göre bu ekstraktların disk difüzyon yöntemine göre bazı mikroorganizmaların büyümesini farklı oranlarda (10-21 mm) engellediği görülmüştür. *T. versicolor*'un kullanılan mikoorganizmalara karşı minimum inhibitör konsantrasyonları. 62.5-250 µg/mL arasında olduğu belirlendi. Metanol ekstraktının TAS ve TOS değerleri sırasıyla 0.72 mmol Trolox Equiv./L ve 18.39 µmol H_2O_2 Equiv./L, etanol ekstraktının TAS ve TOS değerleri sırasıyla 0.88 mmol Trolox Equiv./L ve 16.71 µmol H_2O_2 Equiv./L tespit edildi.

Anahtar kelimeler: Tıbbi mantarlar, *Trametes versicolor*, antimikrobiyal, antioksidan.

1. Giriş

Mushrooms are increasingly appreciated for their medicinal properties as well as their use as functional foods [1]. Since ancient times, mushrooms are benefited in the treatment of many diseases. In many studies conducted today, it has been reported that mushrooms have antioxidant, antimicrobial, antiproliferative, anticancer, DNA protective, antiinflammatory, immunomodulatory and antihypertensive activities [2, 3]. Extracts are prepared from, macrofungi and actinomycetes, which are used in the treatment of many diseases [4]. *Trametes versicolor* (commonly known as turkey tail), which is among the macrofungi, draws attention due to its wide use in the food and pharmaceutical industry [5]. *T. versicolor* (L.) Lloyd, known as turkey tail, is a woody mushroom that grows on different trees such as oak and Prunus, and on different conifers such as fir or pine trees [6]. Although it is a non-edible species, it has traditionally been used in Asia as an alternative source for the treatment of many diseases,

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including cancer and some infections [7]. In our country, *T. versicolor* has commercially been produced by a company since 2000. It is sold in natural form, as ground and sliced in 100 g packages [8]. Generally, 2-5 g of dried mushrooms are boiled in 1 L of water and consumed 2-3 times a day. The tea of this mushroom is used to strengthen immunity, regulate blood sugar, reduce stress and fatigue, anti-aging, increase probiotic microorganisms, reduce oxidative stress of cells and strengthen memory [8].

However, although the medicinal effects of this species are remarkable, its antimicrobial and antioxidant effects are not sufficient in the literature. More emphasis is placed on the anticancer effect of polysaccharides. Therefore, in this study, it was aimed to detect the antimicrobial effect against some pathogenic microorganisms and antioxidant capacity of ethanol and methanol extracts of *T. versicolor*.

2. Materials and Methods

2.1. Material

T. versicolor was collected in April 2017 in Sivrice district of Elazig. The species was identified by performing macroscopic and microscopic studies. After the mushroom was ground, 0.5 g was weighed. 100 mL of 96% methanol (MetOH) and ethanol (EtOH) were added to the samples and left in an orbital shaker at 100 rpm for 72 h. Then, samples were filtered with blank disc.

2.2. Antimicrobial effect

2.2.1. Microorganisms

In this study; *E. coli* ATCC25922, *K. pneumoniae* ATCC700603, *P. aeruginosa* DMS 50071, *B. megaterium* DSM32, *S. aureus* COWAN1, *C. albicans* FMC17 and *Trichophyton sp*. were used. Microorganism were obtained from Firat-University, Department of Biology.

2.2.2. Preparation of microorganism cultures and antimicrobial testing

The antimicrobial activity was determined by the disc diffusion method [9]. Mueller Hinton Agar, Yeast Malt Extract Agar and Sabouraud Dextrose Agar were sterilized separately in an erlen, then cooled to 45-50°C and inoculated with culture of bacteria, yeast and dermatophyta (10^6 cells / mL of bacteria, 10^4 cells / mL yeast and 10^4 cells/mL dermatophyta as per Mc Farland standard). After shaking well, they were poured into sterile petri dishes and homogeneously dispersed. Discs (6 mm diameter) impregnated with 100 µL (1000 µg) of extracts were placed on microorganism-inoculated plates. Then the plates were kept at 4^0C for 2 h. The inoculated petri dishes were incubated at 37 ± 0.1^0C at 24 h for bacteria and at 25 ± 0.1^0C at 72 h for yeasts and dermatophyta fungi. Standard discs were used for bacteria (Streptomycin sulphate 10 µg / disc) and yeasts (Nystatin 30 µg / disc). The antimicrobial effect was detected by measuring the inhibition zone in mm.

2.2.3. The minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of MetOH and EtOH extracts of *T. versicolor* was detected using macro-broth dilution techniques [10]. A two fold serial dilution of the reconstituted extract was prepared in Mueller Hinton Broth. Each dilution was seeded with 100 µL of the standardized suspension of the test organisms and incubated for 24 h at 37°C. All extracts were tested at 1000-15.625 µg/mL concentrations.

2.3. Antioxidant effect

2.3.1. Total antioxidant activity (TAS) and total oxidant activity (TOS)

TAS and TOS levels of MetOH and EtOH extracts of sample were determined with Rel Assay kits (Rel Assay Kit Diagnostics). TAS value was given as mmol Trolox equiv./L and Trolox was used as the calibrator [11]. The TOS value was expressed as µmol H_2O_2 equiv./L and hydrogen peroxide was used as the calibrator [12].

2.3.2. 2.2-diphenyl-1-picrilhydrazyl radical scavenging capacity (DPPH)

The antioxidant activity of different concentrations of MetOH and EtOH extracts of *T. versicolor* was detected according to the DPPH radical scavenging capacity method [13]. The solution was prepared in methanol and ethanol at a concentration of 25 mg/mL of the obtained extract and diluted 4 times to obtain the calibration curve of DPPH. 40 µL of the solution was taken and 160 µL of DPPH solution was added. After mixing well, the plate left in the dark for 30 min. Methanol and ethanol were used as controls. At the end of the period, absorbances of each mixture were read at 570 nm in the spectrophotometer. percent inhibition values were calculated;

% DPPH inhibition = [(AbsControl - AbsSample) / AbsControl] x 100 (1)

2.4. Statistical analysis

The statistical analysis was performed according to the Kruskal Wallis test.

3. Results

3.1. Antimicrobial effect

Antimicrobial activities of *T. versicolor* extracts obtained from MetOH and EtOH against, used microorganisms are given in Table 3.1. According to the results obtained from, methanol and ethanol extracts of *T. versicolor*, it inhibited the development of *C. albicans* the most.

Table 3.1. Inhibition zones of extracts of *T. versicolor* against some microorganisms (mm)

	T. versicolor-MetOH	*T. versicolor*-EtOH	Standard antibiotics
E.coli	15.66 ± 0.33^{ab}	12.33 ± 0.33^{b}	20.21 ± 0.57*
K. pneumoniae.	16.33 ± 0.33^{ab}	21.75 ± 0.46^{ab}	19.13 ± 0.43*
P. aeruginosa	11.66 ± 0.33^{b}	12.33 ± 0.33^{b}	10.33 ± 0.33*
B. megaterium	12.66 ± 0.33^{b}	10.23 ± 0.33^{b}	20.25 ± 0.57*
S. aureus	15.66 ± 0.33^{ab}	10.23 ± 0.33^{b}	20.23 ± 0.33*
C. albicans	26.76 ± 0.46^{ab}	24.74 ± 0.48^{ab}	21.32 ± 0.42**
Tricophyton sp.	24.76 ± 0.46^{ab}	11.26 ± 0.33^{b}	12.24 ± 0.33**

(Streptomycin sulphate *10 µg/disc for bacteria, Nystatin** 30 µg/disc for yeast and dermatophyta), Means in the same column with the different superscript are significantly different ($p<0.05$)

In the results obtained, minimum inhibitory concentration values of extracts against some microorganisms were determined. Minimum inhibitory concentration values between 62.5 -125µg/mL were determined in the MetOH extract. The MIC value of the EtOH extract of *T. versicolor* against microorganisms were detected between 62.5-250 µg/mL (Table 3.2).

Table 3.2. MIC values of extracts of *T. versicolor* against some microorganisms (µg/mL)

Microorganisms	***T. versicolor*-MetOH**	***T. versicolor*-EtOH**
E.coli	62.5	125
K. pneumoniae	62.5	62.5
P. aeruginosa	125	250
B. megaterium	62.5	250
S. aureus	62.5	125
C. albicans	125	250
Tricophyon sp.	125	125

3.2. Antioxidant effect

The TAS and TOS values of the MetOH extract of mushroom was found to be 0.72 mmol and 18.39 µmol, respectively and the EtOH extract of mushroom was 0.88 mmol and 16.71 µmol, respectively (Table 3.3).

Table 3.3. TAS and TOS values of *T. versicolor*

	TAS(mmol Trolox equiv./L)	TOS (µmol H_2O_2 equiv./L)
***T. versicolor*-MetOH**	0.72 ± 0.231	18.39 ± 0.187
***T. versicolor*-EtOH**	0.88 ± 0.235	16.71 ± 0.131

Values are means ±S.D.n:3, $p<0.05$ importantly dissimilar

The percent inhibition of the DPPH of *T. versicolor* is seen in Table 3.4. It was detected that the antioxidant effects of MetOH and EtOH extracts increased with increasing concentration.

Table 3.4. Percent inhibition of the DPPH radical of *T. versicolor*

	***T. versicolor*-MetOH**	***T. versicolor*-EtOH**
1000 µg/mL	62.80 ± 0.231	64.84 ± 0.724
500 µg/mL	50.60 ± 0.724	60.03 ± 0.645
250 µg/mL	26.82 ± 0.440	38.78 ± 0.732
125 µg/mL	10.97 ± 0.635	22.42 ± 0.724

Values are means ±S.D.n:3, $p<0.05$ importantly dissimilar with Kruskal Wallis's test

4. Discussion

Methanol extract of *T. versicolor* was detected to form inhibition zones (24.14-30.18 mm) at different rates against *E. coli, K. pneumonia*, *P. aeruginosa* and *S. aureus* [5]. It was detected that the methanol extract of *T. gibbosa* at 30 mg/mL inhibited the growth of *E. coli*, *S. aureus*, *K. pneumoniae* and *C. albicans* at different rates (zones of inhibition, respectively 19.50, 20.67, 17.00, 20.80 mm) The methanol extract of *T. elegans* (30 mg/mL) showed different antimicrobial effects against similar microorganisms (18.00±0.75-23.50±0.55mm). Methanol extracts of *T. gibbosa* and *T. elegans* have been reported to have MIC ranging from 6 to 20 mg/mL against microorganism used [14]. MIC values of the methanol extract of *T. versicolor* were determined against *E. coli*, *P. aeruginosa and C. albicans* [15]. Inhibition zones of *T. versicolor* extract at 150 µL concentration were found between 7-8 mm against *B. subtilis*, *C. albicans*, *K. pneumoniae* (MDR) and *S. aureus* [16]. Mycelial extracts of *T. versicolor*, *T. gibbosa*, *T. hirsuta* have been reported to inhibit the growth of *C. albicans* at a concentration of 32.0 mg/mL [17]. When the obtained results are compared with previous studies, it is seen that there are differences. The results seem to vary depending on the species used, the habitat of the species, the microorganism, the solvent and most importantly the concentrations [5, 14-17].

Antioxidant effect of the methanol extract of *T. versicolor* at different concentrations was determined in the range of 32.62-72.32% [5]. The IC_{50} values of the DPPH of the water and ethanol extracts of the same species were calculated as 11.9±1.1 and 5.6±0.8 µg/mL, respectively [7]. The IC_{90} values of methanol and aqueous extracts of *T. versicolor* were determined as 178.83 µg/mL and 518.06 µg/mL, respectively [18]. It has been reported that the percent inhibition of DPPH of the ethanol extract of the same species at different concentrations is between 5.26% and 26.77%. In the same study, TAS and TOS values were calculated as 0.820 ± 0.063 mmol Trolox equiv./L and 17.760 ± 0.456 µmol H_2O_2 equiv./L, respectively [19]. The antioxidant effects of *T. versicolor* at 250, 500, 1000 and 5000 µg/mL concentrations were determined as 2.97% ± 0.14, 5.14 ± 0.27%, 8.20 ± 0.40% and 28.6 9± 0.50%, respectively [20]. Study results compared with previous studies, it was determined that some were higher than others [7, 18-20]. The main reason for this might depend on the habitat of the fungus, the time of collection and the concentrations used.

5. Conclusion

It was detected that the methanol extract of *T. versicolor* showed the best antimicrobial activity against *C. albicans*. In addition, methanol extract showed antimicrobial effect at lower concentrations compared to ethanol extract. It was determined that *T. versicolor*'s methanol and ethanol extracts had good TAS values, but high TOS values. Therefore, it can be said that the presence of oxidant compounds in methanol and ethanol extracts of *T. versicolor* is high. It was determined that the scavenging effect of DPPH radical of *T. versicolor* was lower than the controls. It is known that in the region where this species is collected, it is boiled for healing purposes and its water is consumed. We think that *T. versicolor* has little medical effects in literature studies and this study is important in terms of bringing it into the literature. We predict that the results obtained are important in terms of pharmacology and that mushroom extracts can be used as antimicrobial and antioxidant agents.

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